REMARKS

Claims 1 and 3-9 are pending in the present application. Claim 7 is amended. Support for the recitation of "wherein said bactericidal or bateriostatic composition inhibits germination of spores from spore-forming bacteria and koji mold" is found in the specification, such as on page 8, lines 3-15. No new matter is inserted into the application.

Advisory Action

The Examiner has considered and entered into the record the Reply after Final filed on November 22, 2004. However, the Examiner states that the Reply does not place the present application into condition for allowance. Thus, for purposes of Appeal, claims 1 and 3-9 remain rejected.

Interview

An interview was held with the Examiner and Supervisory Examiner David Naff on January 28, 2005. The assistance of the Examiners in advancing prosecution of the present application is greatly appreciated. Applicants respectfully submit that the following remarks fully address the issues discussed during the interview.

Rejection under 35 U.S.C. § 102(b)

Claim 7 stands rejected under 35 U.S.C. § 102(b) for allegedly being anticipated by EP 0 880 894 (hereinafter EP '894). Applicants respectfully traverse. Reconsideration and withdrawal of the instant rejection are respectfully requested.

The Examiner asserts that the composition claimed in claim 7 is identical to the antibacterial composition disclosed in EP '894. Applicants respectfully disagree. EP '894 merely discloses a selective bactericide which inhibits the growth of food-borne pathogenic bacteria. EP '894 fails to disclose an antibacterial substance which inhibits the germination of spores from spore-forming bacteria or koji mold.

Applicants believe that the rejection is the result of a misunderstanding of the present invention. In particular, the Examiner seems to equate an "inhibition of the growth of cells" as taught by EP '894 and an "inhibition of germination of spores" as recited in the instant claim 7, which is not correct. As such, the following explanation of the present invention is presented so that the differences between EP '894 and the instant claims are clear.

<u>The biology of spore-forming bacteria</u>

Spore-forming bacteria <u>proliferate</u> in the form of vegetative cells when they are in a suitable condition for growth (e.g., suitable temperature, enough nutrition, etc.). In contrast, spore-forming bacteria <u>form spores</u> under severe conditions (e.g., high temperature, no nutrition, etc.). However, once favorable conditions are reestablished, spores can germinate to give rise to vegetative organisms. For information on the differences between spores and vegetative cells, the Examiner's attention is directed to the definitions of "spore," "endospore" and "vegetative" extracted from the Dictionary of Microbiology and Molecular Biology (attached hereto as **Exhibit 1**).

<u>"Inhibition of germination of spores"</u>

As described in the present specification, the present invention provides a substance which <u>inhibits germination</u> of spores of the spore-forming bacteria or koji mold. See, for example, page 8, line 10 of the specification. This feature of the present invention is also recited in claim 7. Example 1 of the specification describes the experimental procedure used to show inhibition of spore germination:

> More particularly, the supernatant, 50 µL, was fed in 6 mm diameter holes made on a flat plate of a potato dextrose agar medium (Nissui Seiyaku Kabishiki Kaisha) inoculated with spores of *Bacillus subtilis*, allowed to stand at 15°C for three hours, and then kept at 37°C for 24 hours to allow *Bacillus subtilis* to proliferate. The diameter of proliferation inhibition circles formed around the holes was measured to determine the antibacterial activity against *Bacillus subtilis*.

See, page 10, line 20 to page 11, line 3 of the specification (emphasis added). Prior to the present invention, no substance was found or known in plants that had the function of inhibiting the germination of spores from spore-forming bacteria or koji mold.

"Inhibition of the growth of cells"

In contrast to the present invention, EP '894 fails to teach the inhibition of the germination of spores from spore-forming bacteria or koji mold. Instead, EP '894 merely shows that Stevia extract was not able to kill *Bacillus cereus* completely and that some *B. cereus* survived in spore form.

Specifically, Example 3 of EP '894 shows the bactericidal effect of Stevia extract on pathogenic bacteria. In this Example, vegetative cells of *B. cereus* were incubated with Stevia extract and inoculated on a plate. The number of colony forming units

(cfu) per ml was then counted. Thus, the only bacteria that could be detected by this procedure were vegetative cells.

As shown in Figure 4, the majority (but not all) of the live cells were killed by the Stevia extract. In column 8, lines 4-5, it is noted that a small portion of the *B. cereus* was detected after treatment with a high concentration of the Stevia extract. Some of the *B. cereus* cells were detected because some vegetative cells formed spores to survive and those spores germinated to give rise to vegetative cells. Thus, EP '894 does not demonstrate that inhibition of germination of spores from spore-forming bacteria or koji mold into vegetative cells. Instead, EP '894 merely shows that *B. cereus* formed spores under severe conditions (i.e., incubation with Stevia extract). EP '894 does not show that the spores were inhibited from germination by Stevia extract.

Response to Examiner's Rejection

The Examiner maintains that the teaching of any amount of inhibition of spore-forming bacteria anticipates claim 7. In other words, the Examiner argued during the interview that EP '894 showed at least some of the *Bacillus cereus* was killed, and therefore EP '894 teaches a bactericide that inhibits germination of spores from spore-forming bacteria. Applicants respectfully disagree.

As is obvious from the excerpt of Example 1 above, the substance of the present invention was tested on spores, not vegetative cells, of *B. subtilis*. Example 10 demonstrates that the substance of the present invention inhibits germination of spores. If any growth of cells on the medium were observed in this test, such growth would be evidence that the spores germed to regenerate vegetative cells. Since such growth was not observed, the substance of the present invention is proven as an inhibitor of spore germination. In contrast, EP '894 merely shows that Stevia extract is an inhibitor of the growth of vegetative cells. EP '894 does not disclose or suggest a substance which is an inhibitor of spore germination. As such, EP '894 fails to anticipate the present invention.

In summary, the substance of the present invention inhibits the germination of spores. This function is clearly distinct from that of "inhibition of the growth of cells" as disclosed by EP '894. The Examiner has failed to produce any document which demonstrates that there is a substance in plants which can inhibit germination of spores. For these reasons, withdrawal of the instant rejection is therefore respectfully requested.

Forthcoming Declaration

The Inventor, Dr. Sakai, is currently preparing a Declaration under 37 C.F.R. § 1.132 showing that the substance of the present invention is effective to inhibit germination of spores, but does not inhibit the growth of vegetative cells. The Declaration will be forthcoming in a Supplemental Preliminary Remarks.

Rejection under 35 U.S.C. § 103(a)

Claims 1 and 3-9 stand rejected under 35 U.S.C. § 103(a) for allegedly being obvious over WO 01/07135 (hereinafter WO '135) or U.S. Patent No. 6,063,382 (hereinafter USP '382), in view of Sakai et al. (1990). Applicants respectfully traverse. Reconsideration and withdrawal of the instant rejection are respectfully requested.

As mentioned above, the method of the present invention provides a method for production of an antibacterial substance that inhibits germination of spores from spore-forming bacteria. WO '135 and US '382 disclose the solvent extraction method. It is known that the solvent extraction can disintegrate plant cells and extract the content of plant cells.

However, in the method of the present invention, the tissue of the plant is disintegrated with an enzyme capable of acting on protopectin. Protopectin constitutes middle lamellae and connects

plant cells in the plant tissue. As described on page 2, line 20 to page 3, line 12 of the specification, action of the above enzyme on plant tissue causes the isolation of cells, i.e. those cells become single cells, but are not disintegrated. Therefore, by the method of the present invention, plant cells are not disintegrated, but respective cells are isolated into single cells and substances contained in middle lamellae (between plant cells) are solubilized together with pectins (see page 3, lines 15-22). Thus, the mechanism of production of the antibacterial substance of the present invention is totally different from that of WO '135 or US '382.

Moreover, isolation of plant cells into single cells and obtaining substances between plant cells are not disclosed or suggested in WO '135 or US '382. Sakai et al. (the present inventor) discloses that protopectinase catalyzes the release of pectin from protopectin. This article does not disclose or suggest an antibacterial substance or the presence of the same between plant cells.

Therefore, the present invention is not obvious over the combination of WO '135 or US '382 with Sakai et al. Withdrawal of the instant rejection is therefore respectfully requested.

Conclusion

Applicants respectfully submit that the above remarks fully address and overcome the outstanding rejections. For the foregoing reasons, Applicants respectfully request the Examiner to withdraw all of the outstanding rejections and objections, and to issue a Notice of Allowance indicating the patentability of the present claims. Early and favorable action of the merits of the present application is thereby respectfully requested.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Kristi L. Rupert, Ph.D. (Reg. No. 45,702) at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees

required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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spots' occur e.g. at sites occupied by the modified base 5-methylcytosine (see DNA METHYLATION); spontaneous deamination of this base generates thymine, resulting in a G·C-to-A·T transition. (The thymine residue, being a normal component of DNA, cannot be recognized by any DNA REPAIR system.) Other hot-spots may contain e.g. DIRECT REPEATS or INVERTED REPEATS, and these are apparently associated with insertions or deletions (and hence e.g. FRAMESHIFT MUTATIONS). In *E. coli*, error-prone DNA repair (see SOS SYSTEM) is apparently responsible for a low level of random (non-targeted) mutagenesis. Deletions, insertions, inversions etc may also be attributable to the activities of TRANSPOSABLE ELEMENTS.

[Analysis of spontaneous mutations in the *lacI* gene of *E. coli*: JMB (1986) *189* 273–284.]

spoonleaf A disease of redcurrants caused by the raspberry ringspot virus (a NEPOVIRUS).

spora That fraction of the particulate matter in AIR (q.v.) consisting of, or including, SPORES.

sporabola (*mycol.*) The trajectory of a forcibly-discharged basidiospore.

sporadin The gametocyte form of a gregarine.

sporangiole *Syn.* SPORANGIOLUM.

sporangiolum (sporangiole) (*mycol.*) A SPORANGIUM-like structure containing a single spore or a small number of spores; it characteristically lacks a columella. Some fungi form both sporangiola and sporangia (see e.g. Choanephoraceae in MUCORALES). The sporangiolum may be an evolutionary intermediate between the sporangium and the CONIDIUM.

sporangiomycin See THIOSTREPTON.

sporangiophore A modified or undifferentiated, simple or branched hypha which bears at least one SPORANGIUM or SPORANGIOLUM. In some fungi (e.g. *Pilaira, Pilobolus*) the sporangiophores are positively phototropic.

sporangiospore (*mycol.*) A thin-walled, motile or non-motile spore formed in a SPORANGIUM or in a SPORANGIOLUM; such spores are characteristic of the lower fungi. (cf. CONIDIUM.)

sporangium (1) (*mycol.*) In some lower fungi: a sac-like structure whose contents are converted into motile or non-motile spores (sporangiospores). (cf. MEROSPORANGIUM; SPORANGIOLUM; ZOOSPORANGIUM.) Sporangia are often globose or elongated, and may occur e.g. singly and terminally on a sporangiophore, in clusters on branched sporangiophores, or (e.g. in *Albugo*) in basipetally formed chains; in many fungi (e.g. *Mucor*) the sporangium contains a COLUMELLA. A sporangium may be deciduous at maturity (e.g. in *Dictyuchus*) or it may remain attached to the sporangiophore (e.g. in *Saprolegnia*); the spores may be released via a pore or by dissolution of the sporangial wall. In e.g. *Pilobolus* the entire sporangium is forcibly discharged. (See also *meiosporangium* and *mitosporangium* under ALLOMYCES, and *zygosporangium* under ZYGOSPORE.)

(2) (*bacteriol.*) The cell in which an ENDOSPORE is formed.

(3) (*bacteriol.*) That part of a cell which subsequently develops into an endospore.

(4) (*bacteriol.*) In e.g. *Actinoplanes* and members of the MYXOBACTERALES: a specialized structure containing one or more spores.

spore A differentiated form of an organism which may be (a) specialized for dissemination; (b) produced in response to, and characteristically resistant to, adverse environmental conditions; and/or (c) produced during or as a result of an asexual or sexual reproductive process. (Not all microorganisms can produce spores.) A spore may be unicellular (i.e., it may contain only one protoplast), bicellular, or multicellular; it may be thick-walled or thin-walled, pigmented or non-pigmented, motile or non-motile. Under suitable conditions, disseminative and resistant forms of spore typically give rise to vegetative organism(s); a spore formed in a reproductive process may e.g. give rise to a vegetative organism or act as a gamete.

Bacterial spores. Bacterial ENDOSPORES (q.v.) are resistant (and may be disseminative) forms rather than reproductive forms, while the EXOSPORES formed e.g. by species of the ACTINOMYCETALES (q.v.) are characteristically reproductive and disseminative forms. (See also *myxospores* in MYXOBACTERALES.)

Fungal spores. See e.g. ASCOSPORE; AZYGOSPORE; BALLISTOSPORE; BASIDIOSPORE; CHLAMYDOSPORE; CONIDIUM (and SACCARDOAN SYSTEM); GEMMA; OIDIUM; SPORANGIOSPORE; STATISMOSPORE; THALLOSPORE; ZYGOSPORE. (See also UREDINIOMYCETES and USTILAGINALES.)

Some fungal spores exhibit DORMANCY. *Exogenous* dormancy is that due to the effects of environmental conditions; germination occurs only under conditions favourable for vegetative growth. *Endogenous* (= *constitutive*) dormancy is due to internal factors; these may include (a) the presence of a permeability barrier to nutrients, (b) the existence of a (reversible) metabolic block, and/or (c) the presence of an endogenous chemical inhibitor of germination. The self-inhibitor in e.g. uredospores of *Puccinia graminis* is methyl-*cis*-ferulate (which inhibits removal of the germination plug in the spore wall), while in the uredospores of some other rusts the inhibitor is methyl-*cis*-3,4-dimethoxycinnamate (which blocks initiation of the germ tube). In general, self-inhibitors may account for the low rate of germination of spores in masses; the leaching

of inhibitor may account for the enhancement in germination when such spores are washed with water.

Dormancy can be broken by *activation* e.g. by certain chemical agents (*activators*) that include detergents and organic solvents; by heating the spores to ca. 50–60°C for 10–20 min (e.g. *Neurospora* spp); or by subjecting the spores to cold (e.g. teliospores of *Puccinia graminis*). Some spores can be induced to germinate by damaging the spore wall.

[Physiology and biochemistry of fungal sporulation: ARPpath. (1982) *20* 281–301.]

Protozoal spores. See e.g. ASCETOSPORA; MICROSPORA; MYXOZOA.

spore ball See USTILAGINALES.

spore coat See ENDOSPORE (sense 1 (a)).

spore print (*mycol.*) A deposit of spores formed when the cap of a mature agaric is left, gills-down, on a piece of plain white paper; it is useful e.g. for determining spore colour.

spore stains See e.g. ENDOSPORE STAINS.

spore strip A strip of filter paper, foil etc on which a suspension of endospores (e.g. those of *Bacillus stearothermophilus*) has been allowed to dry; it is used e.g. for monitoring the performance of an AUTOCLAVE. [Commercial spore strip performance: AEM (1982) *44* 12–18.]

sporic meiosis MEIOSIS in a life cycle characterized by an ALTERNATION OF GENERATIONS.

Sporichthya A genus of bacteria (order ACTINOMYCETALES, wall type I) which occur e.g. in soil. The organisms form an aerial mycelium consisting of sparingly-branched hyphae (0.5–1.2 μm in diameter, up to ca. 25 μm in length) which are anchored to the medium by holdfasts; no substrate mycelium is formed. Aerial hyphae divide into coccoid and rod-shaped forms which, in the presence of water, give rise to motile (flagellated) propagules. Growth occurs at room temperatures and at 37°C. Type species: *S. polymorpha*. [Book ref. 73, pp. 101–102.]

sporicide Any chemical agent which inactivates spores (particularly bacterial ENDOSPORES) irreversibly.

sporidesmins Cyclic depsipeptide MYCOTOXINS produced by *Pithomyces chartarum*. Sporidesmins can cause a mycotoxicosis, 'facial eczema', in sheep or cattle grazing pastures contaminated with *P. chartarum*; facial eczema occurs in parts of Australasia, Africa and the USA. Symptoms, which are strongly dose-dependent, include e.g. anorexia, diarrhoea, dehydration, photosensitivity, jaundice, and inflamed oedematous swellings on the lips, face and vulva; death may occur. Thiabendazole has been used to control the fungus in pastures. Sporidesmin and sporidesmin E inhibit the growth of certain bacteria (e.g. *Bacillus subtilis*) and are extremely toxic (at <1 ng/ml) to a range of mammalian cell cultures (an early effect apparently being the disruption of microfilaments in the cells [JCS (1986) *85* 33–46]). [Book ref. 16, pp. 29–68.]

Sporidiales An order of fungi (class USTILAGINOMYCETES) which are typically saprotrophic although the anamorph of at least one species, *Filobasidiella neoformans* (see FILOBASIDIELLA), is a human pathogen; the organisms characteristically occur in a yeast-like (unicellular) form, but most or all species can form a true mycelium or a pseudomycelium. Teliospores are formed by species of *Leucosporidium*, *Rhodosporidium* and SPORIDIOBOLUS but not by members of the FILOBASIDIACEAE.

Sporidiobolus A genus of fungi (order SPORIDIALES) which form budding, yeast-phase cells, pseudomycelium and true mycelium, and which give rise to ballistoconidia and to intercalary or terminal *teliospores*. The teliospores germinate to form haploid basidiospores (in heterothallic species) or diploid basidiospores (in homothallic species) on basidia which may be phragmobasidia or holobasidia. (In both homothallic and heterothallic species, karyogamy occurs in the teliospore; in heterothallic species meiosis occurs during germination of the teliospore.) Carotenoid pigments (e.g. pink, red) are formed. *S. salmonicolor* and *S. pararoseus* (both heterothallic) are the teleomorphs of two species of SPOROBOLOMYCES: *S. salmonicolor* and *S. shibatanus*, respectively. [Book ref. 100, pp. 532–540.]

sporidium (1) A BASIDIOSPORE formed by the rust or smut fungi. (2) In smut fungi: a spore formed by GERMINATION BY REPETITION.

sporistasis The state in which germination and outgrowth of a viable spore are prevented by chemical and/or other factors.

sporoactinomycetes A category within the order ACTINOMYCETALES which includes spore-forming organisms that are characterized by a morphology more complex than that of the NOCARDIOFORM ACTINOMYCETES.

Sporobolomyces A genus of fungi (see SPOROBOLOMYCETACEAE) which form spheroidal, ovoid or elongate budding yeast cells; some species can form pseudomycelium and true mycelium. The cell walls lack xylose, and growth on malt agar is pink, red or orange due to the presence of carotenoid pigments (cf. BULLERA). Ballistospores are formed on hyphal and yeast cells; they are typically bilaterally symmetrical (e.g. reniform or sickle-shaped) and develop obliquely at the tips of branched or unbranched sterigmata. Metabolism is strictly respiratory. NO_3^- is assimi-

NUCLEOLUS which persists, and divides, during mitosis.

endospore (1) A type of SPORE formed *intracellularly* by the parent cell or hypha. (cf. EXOSPORE.)

(a) (*bacteriol.*) Endospores are formed under conditions of nutrient limitation by e.g. species of *Bacillus, Clostridium, Coxiella, Desulfotomaculum, Sporolactobacillus, Sporomusa* and *Thermoactinomyces* (cf. SPOROSPIRILLUM). In general, endospores are considerably more resistant than are vegetative cells to heat, desiccation, antimicrobial agents and radiation; their irreversible inactivation can be ensured only by the procedures used for STERILIZATION (sense 1). Endospores can remain dormant for long periods; viable endospores of *Bacillus* spp estimated to be ca. 500–1000 years old have been recovered from lake sediments. Studies on endospore formation and germination have been carried out mainly with *Bacillus* spp.

Sporulation in Bacillus spp. Prior to sporulation, a growing (vegetative) cell is said to be in *stage 0*. At the onset of sporulation the chromosomal DNA condenses to form a longitudinal *axial filament* (*stage I*). (Some authors consider that sporulation proper starts at stage II.) In *stage II* the cytoplasmic membrane invaginates near one end of the cell, forming an asymmetric septum which divides the cell into two protoplasts; the smaller protoplast (= *forespore, prespore*), which contains a single chromosome, is the precursor of an endospore. In *stage III* the membrane of the larger protoplast invaginates, engulfing the forespore; at this stage the forespore is therefore bounded by two membranes (the outermost membrane being derived from the invaginated region of the larger protoplast) and it lies within the cytoplasm of the larger protoplast, or 'mother cell'. In *stage IV* PEPTIDOGLYCAN is deposited between the two membranes of the forespore, forming the spore *cortex*; the peptidoglycan differs from that in vegetative cell walls in that e.g. it contains fewer cross-links, and in that the lactyl groups of some muramic acid residues form lactam rings by displacing the *N*-acetyl group. In *stage V* the *spore coat* develops as a lamellate protein structure external to the outer membrane of the forespore; the spore coat is rich in e.g. cystine residues, and it is strongly hydrophobic. During this time, calcium dipicolinate (see DIPICOLINIC ACID) accumulates in the protoplast ('core') of the developing endospore, and in *stage VI* the endospore reaches maturity and develops its characteristic resistance to heat etc. In *stage VII* the endospore is released by lysis of the mother cell. (In some species the mature endospore carries a loose outer membrane (*exosporium*) external to the spore coat; the origin and composition of the exosporium appear to be unknown.)

Various enzymes and other products are synthesized at specific stages of sporulation; thus, e.g. certain proteases and antibiotics are secreted at the onset of sporulation, alkaline phosphatase synthesis is associated with stage III, and glucose dehydrogenase appears in stage V. (See also DELTA-ENDOTOXIN.)

Elucidation of the mechanism of sporulation has been helped by the isolation of a number of mutants, each blocked at a specific stage of sporulation. Such *spo* mutants are designated according to the stage beyond which endospore development does not occur; thus, e.g. mutants with *spoII* and *spoIII* mutations do not carry out stages III and IV, respectively. Mutations which affect a given stage but which occur at different loci are designated *spoIIA, spoIIB* etc. No *spoI* mutant has been isolated, but a mutation at any of 8 or 10 *spo0* loci prevents the development of the asymmetrical septum. In general, *spo* mutations have little or no effect on vegetative growth.

Sporulation is initiated on depletion of the source of carbon, nitrogen or phosphorus. The way in which nutrient depletion triggers sporulation is currently unknown, but it is widely believed that the signal, at molecular level, is an intracellular paucity of guanine nucleotides; this idea is supported by the fact that inhibitors of guanine nucleotide synthesis promote sporulation even in the presence of adequate sources of carbon, nitrogen and phosphorus.

During the initiation and progress of sporulation, gene expression is regulated at least partly at the level of transcription, and the relevant genes are transcribed in a specific temporal sequence. In *Bacillus subtilis*, an early event in sporulation is the synthesis of a new, sporulation-specific SIGMA FACTOR, designated σ^{29}, which confers on an RNA polymerase the ability to recognize different promoters; this has prompted the suggestion that, during sporulation, gene expression may proceed in a regular, programmed sequence as a direct or indirect result of the synthesis of different sigma factors at different stages of the process (cf. BACTERIOPHAGE SPO1). Whether or not sporulation involves a cascade of sigma factors, other regulatory influences are probably needed to ensure the correct timing of gene expression. [Endospore formation in *Bacillus*: Book ref. 67, pp. 63–88.]

Dormancy. Freshly formed endospores usually enter a period of DORMANCY in which little or no metabolic activity can be detected

Activation is a (commonly reversible) process in which a dormant endospore prepares for subsequent germination and differentiation into a vegetative cell; it appears to involve changes in the configuration of the endospore macromolecules, presumably early steps in the mobilization of metabolic potential. Activation may be brought about e.g. by sublethal heating, by 'ageing', or by subjecting the endospores to low pH or to certain chemicals.

Germination is an irreversible process in which an endospore becomes metabolically active. The process is largely degradative; it involves e.g. the hydrolysis and depolymerization of certain constituents of the endospore, and is characterized by the release of dipicolinic acid, calcium ions, and the breakdown products of cortical peptidoglycan — and by a loss of resistance to heat etc. Germination requires, or is encouraged by, certain substances (*germinants*) such as L-proline, L-alanine, certain sugars (e.g. glucose), ions (e.g. K^+, Ca^{2+}, Mn^{2+}, Sr^{2+}), surfactants, and calcium–dipicolinic acid chelate; a given germinant may be effective for the endospores of some species but not for those of others. Germination is also commonly affected by factors such as pH, and may be promoted by mechanical damage to the endospore wall. Certain substances (e.g. D-alanine, $NaHCO_3$) inhibit germination of the endospores of certain species of bacteria.

Outgrowth is the process in which a vegetative cell develops from a germinated endospore. (See also MICROCYCLE SPORULATION.)

[Sporulation and germination: Book ref. 164.]

(b) (*cyanobacteriol.*) See BAEOCYTE.

(c) (*mycol.*) See e.g. COCCIDIOIDES, OOSPORIDIUM, RHINOSPORIDIUM, SARCINOSPORON, TRICHOSPORON.

(2) (*mycol.*) (endosporium) The innermost layer of a fungal spore wall.

endospore stains Bacterial endospores stain poorly (or not at all) with simple staining procedures in the cold. They can be stained e.g. by a modified form of ZIEHL–NEELSEN'S STAIN in which ethanol (only) is used for decolorization. In another method (Schaeffer–Fulton stain) an air-dried, heat-fixed smear is flooded with 0.5% (w/v) aqueous malachite green and heated to near-boiling for 5 min; the smear is then washed in running water, counterstained with safranin (0.5% aqueous) for ca. 30 sec, washed again with water, and blotted dry. Endospores stain green, cells red-brown.

endosporium *Syn.* ENDOSPORE (sense 2).

endosporulation In coccidia: SPORULATION which occurs *within* the host. Endosporulation occurs e.g. in all species of *Sarcocystis* and in *Cryptosporidium*, and it has been reported to occur in certain coccidia parasitic in fish (e.g. *Eimeria carpelli*).

ENDO-STAPH See TEICHOIC ACIDS.

Endostelium See PROTOSTELIOMYCETES.

endosymbiont An organism which lives within the cells or tissues of another organism in a symbiotic association (SYMBIOSIS sense 1). (cf. ECTOSYMBIONT.)

Endothia A genus of fungi of the order DIAPORTHALES. *E. parasitica* [ultrastructure: CJB (1982) *61* 389–399] causes CHESTNUT BLIGHT (q.v.). (See also PROTEASES.)

endothrix infection See DERMATOPHYTES.

endotoxic shock A poorly understood, complex syndrome associated with the presence of ENDOTOXIN (sense 1) in the blood; it occurs e.g. in certain patients suffering from Gram-negative bacteraemia. Symptoms include hypotension and other classical signs of shock. The precise mechanism appears to vary e.g. with host species. [Book ref. 95.]

endotoxin (1) A generic term for the LIPOPOLYSACCHARIDES of Gram-negative bacteria; in mammals, endotoxin can act e.g. as a pyrogen, as an inducer of shock, and as a toleragen. The toxicity of endotoxin is apparently due primarily to lipid A, though the polysaccharide moiety appears to contribute e.g. by conferring water-solubility on the lipid; the biological effects of endotoxin in mammals may involve hypersensitivity as well as other reactions. The mechanisms of endotoxin toxicity are incompletely understood. Fever may be due e.g. to the release of 'endogenous pyrogen' by leucocytes and Kupffer cells subjected to endotoxin; the terminal mediators in the febrile response may be PROSTAGLANDINS. [Book refs 92–94.] (See also ENDOTOXIC SHOCK.)

(2) Any microbial toxin which is released only on cell lysis. (See also DELTA ENDOTOXIN.)

endotrophic mycorrhiza See MYCORRHIZA.

Endotrypanum A genus of protozoa (family TRYPANOSOMATIDAE) parasitic in the erythrocytes of sloths (in Central and South America) and in sandflies (*Lutzomyia* spp) — which transmit the protozoon to sloths. One species (*E. schaudinni*) forms intraerythrocytic epimastigotes, the other (*E. monterogei*) forms intraerythrocytic trypomastigotes; in culture both species form promastigotes.

endotunica See BITUNICATE ASCUS.

endoxylanase See XYLANASES.

endozoite (tachyzoite) In certain coccidia: a stage within host tissues (see e.g. TOXOPLASMOSIS).

energy charge (EC) See ADENYLATE ENERGY CHARGE.

energy-transducing membrane Any biological membrane which contains components that

llus by a septum. After release of the spermatozoids and fertilization, the zygote develops a thick wall and remains dormant for some time; meiosis is believed to occur on germination.

Vauchomia See PERITRICHIA.

VCN agar *Syn.* THAYER–MARTIN AGAR.

VD VENEREAL DISEASE.

VD-1827 SULTAMICILLIN.

VDAC Voltage-dependent anion channel: a PORIN channel in the mitochondrial outer membrane.

VDRL test (Venereal Disease Research Laboratory test) A qualitative or quantitative STANDARD TEST FOR SYPHILIS. A fixed volume of the test antigen is added to a fixed volume of the patient's INACTIVATED SERUM (or a known dilution of it) on a slide; mixing is conducted under standard conditions for a fixed period of time. Flocculation (aggregation of the antigen particles into clumps) occurs in the presence of reagins, and is detected under the microscope (×100); large clumps are reported as 'reactive', small clumps as 'weakly reactive', and a regular or finely granular suspension of antigen as 'non-reactive'. Strongly positive sera may exhibit a PROZONE.

vector (1) (*med.*, *vet.*, *plant pathol.*) Any living organism — conventionally, an invertebrate, or a microorganism (see e.g. PLASMODIOPHOROMYCETES) — which effects the transmission of a parasite (e.g. a pathogenic bacterium, fungus, protozoon or virus) from one individual (man, animal or plant) to another. (cf. CARRIER; VEHICLE.) In many cases a parasite can be transmitted by only one or a few species of vector; in other cases there may be little or no parasite–vector specificity. Common vectors of human and animal parasites include flies, lice, mosquitoes and ticks, while vectors of plant parasites include insects such as aphids, leafhoppers, and whiteflies, as well as mites, fungi (see e.g. OLPIDIUM), nematodes, etc.

In a *biological vector* the parasite replicates and/or undergoes one or more stages of development in the vector (see e.g. CYCLICAL TRANSMISSION and *propagative* CIRCULATIVE TRANSMISSION). In a *mechanical vector* the parasite is transported more or less passively by the vector (cf. NON-CIRCULATIVE TRANSMISSION).

(See also TRANSOVARIAL TRANSMISSION and TRANSSTADIAL TRANSMISSION.)

(2) (*mol. biol.*) See CLONING.

vectorial electron transfer See EXTRACYTOPLASMIC OXIDATION.

vectorial group translocation Directional translocation of given molecular or ionic species across a membrane as a consequence of the presence of fixed pathways across the membrane; in this concept, translocation of the species does not involve conformational changes in the membrane proteins — the latter merely providing, passively, suitable transmembrane routes. This concept is exemplified in the LOOP MODEL of proton translocation by the respiratory chain (see ELECTRON TRANSPORT CHAIN and CHEMIOSMOSIS).

VEE VENEZUELAN EQUINE ENCEPHALOMYELITIS.

vegetable spoilage See e.g. CANNING; GANGRENE (2); PECTIC ENZYMES; PICKLING; SOFT ROT (2).

vegetative (1) (assimilative; somatic; trophic) Refers to a cell or organism, or to a stage in an organism's life cycle, in which nutrition and growth (as opposed to e.g. sexual reproduction or dormancy) predominate.

(2) (*virol.*) Refers to the 'growth phase' of a virus (i.e., the phase in which progeny virions are formed), or to a virus in the 'growth phase'.

vegetative mycelium (in actinomycetes) *Syn.* SUBSTRATE MYCELIUM.

vehicle (1) (*epidemiol.*) An inanimate medium in or on which a (usually pathogenic) microorganism may be transmitted: e.g., unpasteurized milk. (cf. VECTOR.)

(2) (*mol. biol.*) *Syn.* cloning vector (see CLONING).

(3) (*mol. biol.*) The organism in which a recombinant DNA molecule is replicated during CLONING experiments.

veil (*mycol.*) See PARTIAL VEIL and UNIVERSAL VEIL.

Veillon tube A glass tube (e.g. 25 cm long, internal diam. 1 cm) which can be sealed at both ends with rubber bungs; it is used for SHAKE CULTURE (sense 1).

Veillonella A genus of Gram-negative bacteria (family VEILLONELLACEAE) which occur e.g. in the mouth and in the intestinal and respiratory tracts of man and other animals. Cells: cocci, 0.3–0.5 μm diam., often in pairs. Some species (*V. criceti*, *V. dispar*, *V. ratti*) form a pseudocatalase. Colonies fluoresce red under ultraviolet light (360 nm). The organisms ferment e.g. lactate (forming acetate, propionate, CO_2 and H_2), fumarate and pyruvate; in general, carbohydrates are attacked weakly or not at all. GC%: (Bd) ca. 40–44. Type species: *V. parvula*. Other species: *V. atypica*, *V. caviae*, *V. rodentium*. (*V. alcalescens* is currently included within *V. parvula*.)

Veillonellaceae A family of Gram-negative, oxidase-negative, chemoorganotrophic, anaerobic bacteria which occur e.g. in the intestinal tract in man, ruminants and other animals. Cells: non-motile cocci, ca. 0.3–2.5 μm diam., which usually occur in pairs (adjacent sides of cells may be flattened); cells may resist decolorization in the Gram stain. No species forms catalase, but some strains form